Antineoplastic Activity of *Bis*-tyrosinediaqua Ni(II) Against Ehrlich Ascites Carcinoma

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ABSTRACT: In order to find out new compounds having antineoplastic activity, *bis*-tyrosine diaqua nickel (II) complex was synthesized and characterized. The antineoplastic activity of the compound was judged by measuring inhibition of tumour growth and enhancement of survival time of EAC cell bearing mice. In addition its effects on haematological parameters and serum alkaline phosphatase activity of both normal and EAC bearing mice were also studied. The test compound significantly inhibited the tumour growth and increased life span of tumour bearing mice at dose 10 mg/kg i.p. The treatment also recovered the perturbed haematological parameters as well as the serum alkaline phosphatase activity towards normal. The host toxicity of the test compound was found to be negligible. The compound can be considered as an effective antineoplastic agent.

Key words: Antineoplastic activity, *Bis*-tyrosinediaqua nickel (II), EAC, ALP activity

INTRODUCTION

Researches involving metal complexes have been extensively carried out[1–4] owing to their potential applications in biological and pharmaceutical fields. Antineoplastic activities of various complexes of platinum, nickel, copper, zinc etc. are well known.[3,5,6] Europium(III) complexes with some schiff bases are found to be effective[7] against hepatic carcinoma (HCT8) and leukemia cells (L_{210}). Vanadium complexes with salicylaldehyde semicarbazone derivatives can be used[8] as antineoplastic agent towards kidney tumour cells (TK10). The inhibiting ability on cell proliferation (MCF human breast cancer cell) of nickel(II) complexes of naphthaquinone thiosemicarbazone and semicarbazone have also been reported recently.[9] The present study has been focused on the utilization of a nickel complex with tyrosine to evaluate its efficiency in cell growth inhibition and enhancement of life span of tumour bearing mice inoculated with ehrlich ascites carcinoma (EAC) cells. The study has been extended to examine also the effect of the compound on some perturbed haematological parameters and alkaline phosphatase activity of tumour bearing mice.

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MATERIALS AND METHODS

Chemicals. All chemicals were purchased from BDH (England) and used without further purification.

Animal. Adult Swiss Albino male mice of 6–8 weeks of age weighing 20–25 grams were used throughout the study.

Tumour cells. Ehrlich ascites carcinoma (EAC) cells were obtained through the courtesy of Indian Institute of Chemical Biology (IICB), Kolkata-32, India. EAC cells were maintained every twelve days intraperitoneal inoculation of 1.4×10^5 cells/mouse.

Synthesis of *bis*-tyrosinediaqua nickel(II). The complex was prepared by the method as described in the literature.[4]

Characterization. Elemental analysis of the test compound for carbon, hydrogen and nitrogen was done by using Perkin Elmer 2400 CHN elemental analyzer. Infrared spectra as KBr disc were recorded with Shimadzu FTIR 8101. The percentage of nickel was determined by using an atomic absorption spectrometer (Shimadzu Japan) and TG thermogram was taken with a thermoanalyser of Mettler Instrument Corp. (Highstown, N.J.).

LD_{50} determination. The median lethal dose (LD_{50}) of the test compound was evaluated following conventional methods.[10] The complex solution in 2% DMSO was administered at different doses (i.p) in male mice. LD_{50} was evaluated by recording the mortality up to 24 hours.

Inhibition of tumour growth. EAC cells (1.4×10^5) were inoculated into 4 groups of mice (6 in each) on day 0. Mice of two groups were treated with *bis*-tyrosinediaqua nickel (II) at doses 5 mg/kg i.p and 10 mg/kg i.p respectively. The third group was treated with *bleomycin* at dose 0.3 mg/kg i.p. The control group was treated with the vehicle only. Treatments were continued for 4 days and on day 5 animals were sacrificed. Tumour cells were collected by repeated washing with 0.9% saline and viable tumour cells were counted (Trypan blue test) with a haemocytometer. Total number of viable cells per animal of the treated groups was compared with those of control group.

Survival time. Four groups of mice (n=6) were inoculated (i.p) with 1.4×10^5 cells/mouse on day 0. Treatment with the test compound was started 24 hours after inoculation at doses 10 mg/kg i.p (Group-A), 5 mg/kg i.p (Group-B) and 0.3 mg/kg i.p with *bleomycin* (Group-C) per day. The control group (Group–D) was treated with the same volume of saline 0.9%. Treatment was continued for 10 days. Mean survival time (MST) for each group was noted. Survival time for treated group was compared with those of control group (Group–D) using the following calculation.

$$\text{Increase of life span (ILS)} = \left[\frac{\text{MST of treated group}}{\text{MST of control group}} - 1\right] \text{ x } 100$$

$$\text{Where, MST} = \frac{\sum \text{Survival time (day) of each mouse in a group}}{\text{Total number of mice}}$$

Bioassay of EAC cells. The procedure was a modification of the method as described by Fernandes and Klubes.[11] Two groups of mice (n=4) were inoculated with 2.0×10^5 EAC cells. Group-1 was treated with the nickel complex at the dose of 10 mg/kg i.p. for three consecutive days and group-2 received the vehicle only (served as control). On day 4, tumour cells from the mice were harvested in cold (0.9%) saline, pooled, centrifuged and reinoculated (2×10^5 cells/mouse) into two fresh groups of mice (n=4) as before. No further treatment was done on these mice. On day 5, they were sacrificed and viable tumour cell count/mouse was performed.

Haematological studies. Haematological parameters were studied with the test compound at doses 10 mg/kg i.p and 5 mg/kg i.p both in normal and tumour bearing mice following usual method.[12]

Alkaline phosphatase activity (ALP). ALP activity of the serum of normal and tumour bearing mice treated with the complex (10 mg/kg i.p. and 5 mg/kg i.p. 10 days) was assayed on day 12. The ALP activity was measured according to the procedure of Telfer[13] using paranitrophenyl phosphate (PNPP) as substrate, in glycine sodium hydroxide buffer (pH 10). Absorbance was measured at 410 nm. Unit of enzyme activity is μmol of PNPP hydrolyzed min^{-1} mL^{-1} of serum.

Statistical analysis. The student t–test was used for the statistical analysis of the results. P values < 0.001 were considered as significant.

RESULTS AND DISCUSSIONS

IR (KBr) spectrum of the test compound showed a broad absorption at 3600–3200 cm^{-1} for the combined effect of the presence of –OH, $–NH_2$ groups and H_2O molecules. Further the sharp band at 1710 cm^{-1} is due to C=O group and bands at 530 cm^{-1} and 350 cm^{-1} for Ni-O and Ni–N bonds respectively. The band at 1600 cm^{-1} is due to the presence of aromatic groups. Melting point of the compound could not be measured because of its decomposition after 240°C. About 8.2% weight loss at 120°C - 140°C was found (due to the evaporation of water molecules) from TG analysis. All these data including those from elemental analysis (Table 1) are in accordance with the formula shown in the Figure 1.

Table 1. Analytical data for elements in nickel (II) complex (w/w percentage)

Element	C	H	N	Ni
Experimental value	47.18	5.09	6.27	12.82
Theoretical value	47.50	5.28	6.16	12.91

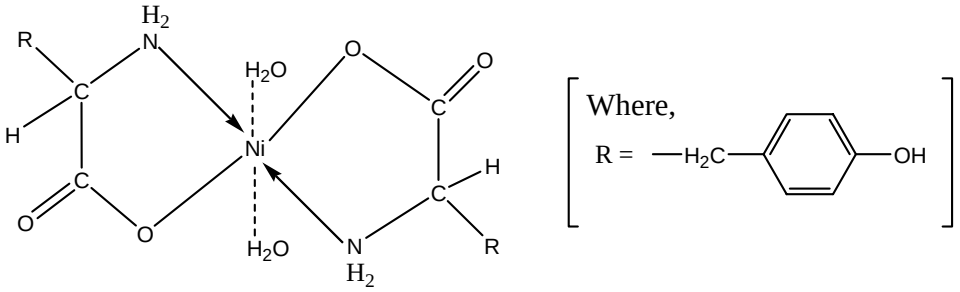

Figure 1. Structure of *bis*-tyrosinediaqua nickel(II) complex

LD_{50} value of *bis*-tyrosinediaqua nickel (II) complex was found to be 50 mg/kg i.p. Treatment with the complex (10 mg/kg i.p. 4 days) resulted in significant (P<0.001) tumour growth inhibition (Figure 2) and increase of the life span of tumour bearing mice (Figure 3). The effect of the nickel(II) complex treatment on the transplantibility of tumour cells was observed by 56% reduction of intraperitoneal tumour burden in mice.

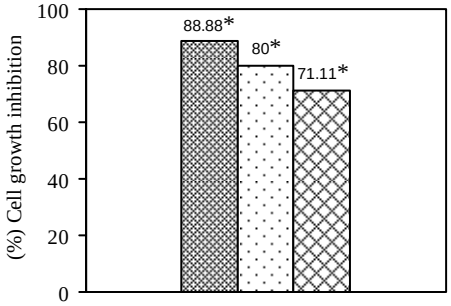

Figure 2. Effect of *bis*-tyrosinediaqua nickel (II) complex on *in vivo* cell growth inhibition
*P < 0.001, when compared with control
Treated with *bleomycin* [0.3 mg/kg (i.p)]
Treated with Ni(II) complex [10 mg/kg (i.p)]
Treated with Ni(II) complex [5 mg/kg (i.p)]

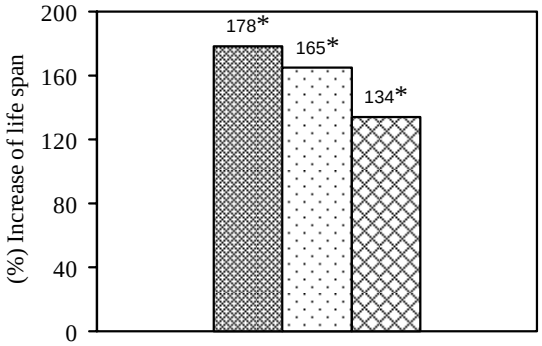

Figure 3. Effect of *bis*-tyrosinediaqua nickel(II) complex on animal's life span
*P < 0.001, when compared with control.
Treated with *bleomycin* [0.3 mg/kg (i.p)]
Treated with Ni (II) complex [10 mg/kg (i.p)]
Treated with Ni (II) complex [5 mg/kg (i.p)]

Haematological parameters of tumour bearing mice on day 12 were found to be significantly altered from those of normal group (Table 2). The total WBC count was found to be increased with a reduction of haemoglobin content of RBC. The total number of RBC showed a significant change. In differential count of WBC, the percentage of

neutrophils increased while the lymphocyte count decreased. At the same time interval the Ni(II) complex (10 mg/kg i.p. 10 days) treatment could restore haemoglobin and RBC values to normal. The increased WBC counts and differential counts were also found to be altered towards normal following the Ni(II) complex treatment. Practically no alteration of haematological parameters in the Ni(II) complex (10 mg/kg i.p. 10 days) treated normal mice was observed (Table 2).

Table 2. Effect of *bis*-tyrosinediaqua Ni(II) complex on haematological parameters in normal and tumour bearing mice

Experiment	Hb (gm dL^{-1})	RBC mL^{-1} $\times 10^{-9}$	WBC mL^{-1} $\times 10^{-6}$	Lymphocyte %	Neutrophil %	Monocyte %
Normal mice (control)	13.75 ± 0.17	8.01 ± 0.48	6.9 ± 0.17	70 ± 0.24	23 ± 2.0	6.0 ± 0.25
EAC bearing mice	8.45 ± 0.50	2.87 ± 0.29	21.0 ± 0.38	44 ± 0.33	40 ± 1.2	8.0 ± 0.47
EAC + Ni (II) complex, (10 mg/kg i.p.)	12.35 ± 0.56*	6.27 ± 0.53*	11.0 ± 0.21*	58 ± 0.25*	26 ± 1.0*	6.5 ± 0.38*
EAC + Ni (II) complex, (5 mg/kg i.p.)	10.15 ± 0.34	4.06 ± 0.30	13.5 ± 0.31	53 ± 2.0	30 ± 1.2	7.0 ± 1.70
Normal + Ni (II)complex, (10 mg/kg i.p.)	12.00 ± 0.25	6.79 ± 0.61	9.0 ± 0.49	78 ± 0.98	23 ± 0.8	14.0 ± 0.98
Normal + Ni (II)complex, (5 mg/kg i.p.)	11.80 ± 0.15	7.10 ± 0.41	7.8 ± 0.20	75 ± 0.52	22 ± 1.2	12.0 ± 0.58

Results are shown as mean values ± SEM. Number of mice per group was 4. *P < 0.001 when compared with control

Growth of EAC cells in mice showed a depletion of ALP activity in serum. Significant recovery of the enzyme activity was found in the serum of tumour bearing mice following the Ni (II) complex (10 mg/kg i.p., 10 days) treatment (Table 3).

Table 3. Effect of *bis*-tyrosinediaqua nickel(II) complex on alkaline phosphatase (ALP) activity in serum of normal and tumour bearing mice

Treatment	Enzyme activity [µmol PNPP hydrolysed min^{-1} mL^{-1} serum]
Normal mice	0.14 ± 0.04
EAC bearing mice	0.05 ± 0.01
EAC + Ni (II) complex (10 mg/kg i.p)	0.11 ± 0.002*
EAC + Ni (II) complex (5 mg/kg i.p)	0.09 ± 0.02
Normal + Ni (II) complex (10 mg/kg i.p)	0.13 ± 0.01
Normal + Ni (II) complex (5 mg/kg i.p)	0.11 ± 0.01

Results are shown as mean values ± SEM. Number of mice per group was 4. *P < 0.001 when compared with control

Results presented in this study show that bis-tyrosinediaqua nickel (II) complex (10 mg/kg i.p., 10 days) inhibits growth of EAC cells significantly in mice and also enhances their life span. The bioassay experiment shows reduction of transplantability of EAC cells treated with the Ni (II) complex indicating loss of viability of the treated cells. Perturbation of haematological parameters in tumour bearing mice is partly responsible for the toxic effects produced in them. In addition myelosuppression in cancer chemotherapy is a common phenomenon that is responsible for poor prognosis.[14] The Ni (II) complex treatment inhibits tumour cell growth, enhances survival of treated mice and restores the haematological parameters. Depletion of alkaline phosphatase activity in tumour bearing mice is also found to be restored by the Ni (II) complex treatment. The observations described above show the efficiency of *bis*-tyrosinediaqua nickel (II) with the above mentioned dose (10 mg/kg i.p.). The compound has practically no adverse side effect on the host.

CONCLUSION

Based on these informations, it can be concluded that, *bis*-tyrosinediaqua nickel (II) is an effective antineoplastic agent and can be utilized in combination with other suitable drugs to protect the host haematological parameters.

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